Received: 1 February 2024 | Revised: 12 March 2024 | Accepted: 29 March 2024

DOI: 10.1002/ajmg.a.63627

AMERICAN JOURNAL OF medical genetics PART A WILEY

CASE REPORT

Immunological and hematological findings as major features in a patient with a new germline pathogenic *CBL* variant

Emilia Stellacci[1] | Jennefer N. Carter[2,3] | Luca Pannone[4] | David Stevenson[2,3] | Dorsa Moslehi[2] | Serenella Venanzi[1] | Undiagnosed Diseases Network | Jonathan A. Bernstein[2,3] | Marco Tartaglia[4] | Simone Martinelli[1]

[1]Department of Oncology and Molecular Medicine, Istituto Superiore di Sanità, Rome, Italy

[2]Stanford Center for Undiagnosed Diseases, Stanford University, Stanford, California, USA

[3]Department of Pediatrics – Medical Genetics, Stanford University School of Medicine, Stanford, California, USA

[4]Molecular Genetics and Functional Genomics Research Unit, Ospedale Pediatrico Bambino Gesù, IRCCS, Rome, Italy

Correspondence
Simone Martinelli, Department of Oncology and Molecular Medicine, Istituto Superiore di Sanità, 00161 Rome, Italy.
Email: simone.martinelli@iss.it

Funding information
Associazione Italiana per la Ricerca sul Cancro, AIRC, Grant/Award Number: IG 28768; NIH Common Fund, Grant/Award Number: U01HG010218; European Joint Programme on Rare Diseases, EJP-RD: NSEuroNet; Italian Ministry of Health

Abstract

Casitas B-lineage lymphoma (*CBL*) encodes an adaptor protein with E3-ligase activity negatively controlling intracellular signaling downstream of receptor tyrosine kinases. Somatic *CBL* mutations play a driver role in a variety of cancers, particularly myeloid malignancies, whereas germline defects in the same gene underlie a RASopathy having clinical overlap with Noonan syndrome (NS) and predisposing to juvenile myelomonocytic leukemia and vasculitis. Other features of the disorder include cardiac defects, postnatal growth delay, cryptorchidism, facial dysmorphisms, and predisposition to develop autoimmune disorders. Here we report a novel *CBL* variant (c.1202G>T; p.Cys401Phe) occurring *de novo* in a subject with café-au-lait macules, feeding difficulties, mild dysmorphic features, psychomotor delay, autism spectrum disorder, thrombocytopenia, hepatosplenomegaly, and recurrent hypertransaminasemia. The identified variant affects an evolutionarily conserved residue located in the RING finger domain, a known mutational hot spot of both germline and somatic mutations. Functional studies documented enhanced EGF-induced ERK phosphorylation in transiently transfected COS1 cells. The present findings further support the association of pathogenic *CBL* variants with immunological and hematological manifestations in the context of a presentation with only minor findings reminiscent of NS or a clinically related RASopathy.

KEYWORDS
CBL, CBL syndrome, Noonan syndrome, phenotypic spectrum, RAS signaling

1 | INTRODUCTION

The Casitas B-lineage lymphoma proto-oncogene (*CBL*; MIM #165360) encodes a widely expressed RING finger E3 ubiquitin ligase involved in the control of cell response to growth factors, hormones, and cytokines (Dikic & Schmidt, 2007; Joazeiro et al., 1999; Swaminathan & Tsygankov, 2006). CBL has a complex role in intracellular signaling by negatively controlling the activity of receptor tyrosine kinases (RTKs) targeting their degradation, and positively modulating signal flow through its adaptor function (Ogawa et al., 2010). Somatic *CBL* mutations occur in myeloid malignancies, particularly juvenile myelomonocytic leukemia (JMML; MIM

Emilia Stellacci and Jennefer N. Carter contributed equally to this study. Marco Tartaglia and Simone Martinelli jointly coordinated this study.

Members of the Undiagnosed Diseases Network are listed in the Acknowledgements.

#607785), and are commonly found as homozygous defects, as a result of acquired uniparental isodisomy of chromosome 11q23 (Hecht et al., 2022; Kales et al., 2010). In 2010, our group and others identified germline heterozygous *CBL* variants in patients affected by a developmental disorder resembling, in part, Noonan syndrome (NS; MIM #PS163950) and predisposing to JMML and cerebral vasculitis (*CBL* mutation-associated syndrome [MIM #613563]) (hereafter, CBL syndrome) (Martinelli et al., 2010; Niemeyer et al., 2010; Pérez et al., 2010). This condition is also characterized by a predisposition to develop autoimmune disorders (Ali et al., 2020; Guey et al., 2017; Niemeyer et al., 2010; Wiel et al., 2020). Due to the impact of *CBL* mutations on RAS-MAPK signaling, CBL syndrome is listed among the RASopathies, a family of developmental disorders that share upregulation of this signaling pathway as a common pathogenic mechanism (Tartaglia & Gelb, 2010).

In contrast to what has been observed for other genes mutated in RASopathies and cancer (e.g., *PTPN11*, *KRAS*, *NRAS*, *BRAF*), the molecular spectra of somatic and germline *CBL* variants largely overlap. The vast majority of disease-causing mutations are in-frame splice site variants or missense changes affecting the RING finger domain or the linker helix region (LHR) linking this domain to the *N*-terminal tyrosine kinase binding (TKB) domain. Consistent with data collected on somatic mutations occurring in cancer (Martinelli et al., 2012; Sanada et al., 2009; Sargin et al., 2007), upregulation of the RAS-MAPK and PI3K-AKT signaling cascades associated with germline variants results from a dominant-negative behavior on CBL-mediated RTKs ubiquitination (Belizaire et al., 2022; Martinelli et al., 2010, 2015; Niemeyer et al., 2010).

CBL syndrome is characterized by wide phenotypic heterogeneity and variable expressivity. Here, we report a novel germline *CBL* variant identified in a subject presenting with minor signs of NS and mostly characterized by hematological and immunological manifestations. Functional studies revealed upregulation of the RAS-MAPK signaling pathway.

2 | METHODS

As detailed in the Supplements, the index patient was enrolled at the Stanford Center for Undiagnosed Diseases in the frame of the Undiagnosed Diseases Network (UDN) program. Genome sequencing was performed as described (Borja et al., 2022). The structure of CBL complexed with a substrate peptide (PDB 4A49) (Dou et al., 2012) was used to predict the functional relevance of Cys^{401}. The c.1202G>T change was introduced in the *CBL* cDNA by site-directed mutagenesis. Constructs were transiently transfected into COS-1 cells, and ERK and AKT phosphorylation was evaluated basally and following EGF stimulation, as previously described (Martinelli et al., 2010, 2012).

3 | RESULTS

3.1 | Clinical report

The patient is a 5-year-old male born at 37 weeks to nonconsanguineous parents. Growth parameters at birth were: weight 2.6 kg (7th centile), length 47.5 cm (18th centile), head circumference 32 cm (4th centile). Within the first few months of life, he developed severe feeding difficulties and recurrent hypertransaminasemia (aspartate transaminase 154–252 U/L, reference values: 8–60 U/L at 1 year [reference range is not established for individuals <1 year]; alanine transaminase 310–363 U/L, reference values: 30–65 U/L). He had two episodes of significant thrombocytopenia, the first at 10 months of age in the setting of fever (platelet count: 131 TH/mm^3; ref. 150–400 TH/mm^3), and the second at 22 months in the setting of hepatitis (platelet count: 130 TH/mm^3) after cephalexin therapy for periorbital cellulitis. Bone marrow studies did not provide any evidence of malignancy, even though a transient monocytosis/JMML-like disorder could not be ruled out. An investigation into immunologic abnormalities, due to prolonged cytomegalovirus (CMV) infection during the first year of life, was unremarkable. He also had a history of frequent illnesses and intermittent petechial rash. Concerns about developmental delay (DD) were first raised at 8–9 months of age when he was not sitting up. He walked at 18 months of age and spoke his first word at 2 years. He was diagnosed with autism spectrum disorder (ASD) at 2 years. At that age, abdominal ultrasound showed mild hepatosplenomegaly. There was no concern for seizures, and no cerebrovascular findings or other abnormalities were noted by brain magnetic resonance imaging (MRI). Currently, he still has difficulties with speech and his expressive language is significantly behind his receptive language.

Physical examination was notable for the presence of multiple café-au-lait macules that continued to increase in number over time (seven typical, neurofibromatosis type I [NF1]-like spots with a diameter >0.5 cm at 4 years 5 months), epicanthal folds, and a low anterior hairline. No other features and signs reminiscent of NS were noted, including hypertelorism, ptosis, down-slanting palpebral fissures, low-set/posteriorly rotated ears, and wide nasal bridge, as well as skeletal and cardiac defects. Growth parameters at 4 years 5 months were: height 112 cm (93rd centile) (mother, 174 cm; father, unknown); weight 22.5 kg (97th centile). At 3 years 11 months, head circumference was 50.5 cm (38th centile). Ophthalmology and audiology evaluations were normal. Metabolic work up was unremarkable.

3.2 | Molecular findings

Trio-based clinical genome sequencing identified a heterozygous variant in the *CBL* gene, c.1202G>T (RefSeq NM_005188.4), predicting the p.Cys401Phe amino acid substitution. No other clinically relevant variants were identified. The missense substitution was not observed in either parental sample, supporting its *de novo* origin (Figure S1). Since this change was not reported in the Genome Aggregation Database (gnomAD, https://gnomad.broadinstitute.org/), nor has it ever been identified as a somatic event in cancer (COSMIC database, https://cancer.sanger.ac.uk/cosmic) or as a published germline event in CBL syndrome, it was initially considered a variant of uncertain significance, and the clinical features of the patient were not considered as fitting the phenotypic spectrum associated with pathogenic

CBL variants to establish a definite diagnosis. However, based on the evidence that Cys^{401} lies in the RING finger domain, a well-known mutational hotspot in *CBL*, and that other substitutions involving the same residue had been reported as somatic events in cancer (Muramatsu et al., 2010; COSMIC database), the variant was re-evaluated through the UDN and considered as "likely pathogenic," according to the ACMG criteria (Richards et al., 2015).

3.3 | Functional validation analyses

CBL is characterized by an *N*-terminal SH2-containing TKB domain, followed by the LHR and RING domain, a proline-rich region and a *C*-terminal portion mediating dimerization and protein–protein interactions (Schmidt & Dikic, 2005; Swaminathan & Tsygankov, 2006). The TKB domain mediates substrate specificity by binding to proteins containing phospho-tyrosine motifs, while the LHR and RING domains play a major role in mediating target ubiquitination. Mutations within the LHR and RING domain, as those observed in patients with CBL syndrome or JMML, abrogate CBL's E3 activity (Martinelli et al., 2010; Niemeyer et al., 2010; Pérez et al., 2010). Specifically, phosphorylation of Tyr^{371}, a CBL mutational hot spot, within the LHR regulates E3 activity by promoting LHR conformational changes that allow the release of the basally autoinhibited conformation of the protein (Dou et al., 2012). The structure of the RING domain is stabilized by two zinc atoms that are coordinated by seven cysteine residues and a single histidine residue (Figure 1a). Mutations affecting these residues have been demonstrated to affect the proper folding of the domain and CBL function

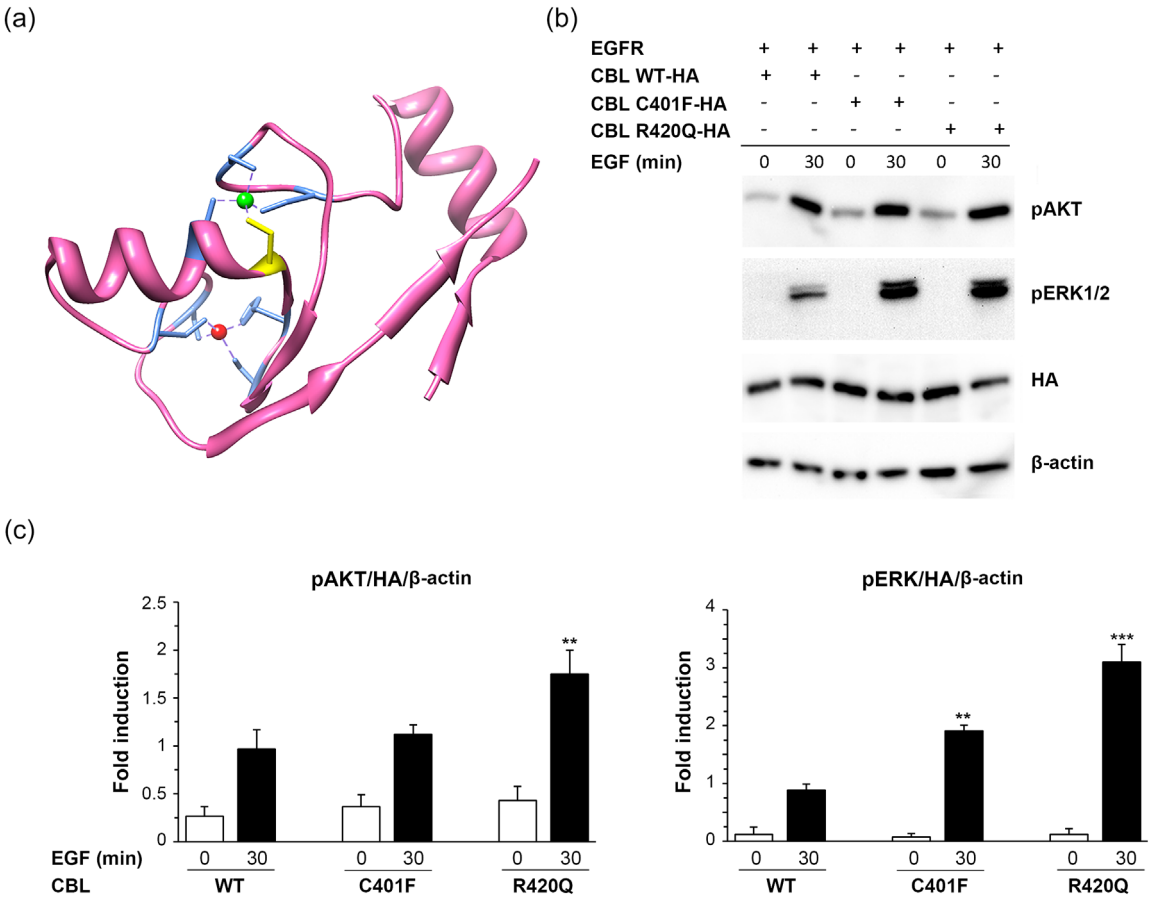

FIGURE 1 Structural and functional consequences of the p.Cys401Phe change in Casitas B-lineage lymphoma (CBL). (a) Location of Cys^{401} within the RING domain of CBL (PDB 4A49). The four residues (Cys^{381}, Cys^{384}, Cys^{401}, Cys^{404}) coordinating the first zinc atom (green), and those (Cys^{396}, $His^{398}Cys^{416}$, Cys^{419}) coordinating the second zinc atom (red) are shown. The mutated residue, Cys^{401}, is highlighted in yellow. Its substitution is predicted to affect the proper folding of the domain. (b) Functional consequences of p.Cys401Phe on intracellular signaling. AKT and ERK phosphorylation assays in COS-1 cells transiently expressing the CBL p.Cys401Phe and p.Arg420Gln mutants, or the wild-type protein. Protein phosphorylation (pAKT, Cell Signaling, #9271; pERK1/2, Cell Signaling, #9106) was evaluated basally or following EFG stimulation (100 ng/mL; 30 min). Equal amounts of cell lysates were resolved on 10% polyacrylamide gel. β-Actin (Sigma-Aldrich, #A5316) and CBL (anti-HA-HRP, Roche, #12013819001) levels are also shown for equal protein expression and loading. Representative blots of four independent experiments are shown. (c) Activation of AKT and ERK1/2 is expressed as a multiple of basal activation in cells transfected with wild-type CBL. Mean densitometry values ± *SD* of the four independent experiments are shown. Asterisks indicate significant differences compared with wild-type CBL at the corresponding time point, basally or upon EGF stimulation (**$p < 0.01$; ***$p < 0.001$; two-way analysis of variance followed by Tukey's multiple comparison test). A single letter code is used to specify amino acid substitutions.

(Ota et al., 2000). As shown, Cys^{401} is one of these key residues that stabilize the RING domain contributing to the coordination of one of the zinc atom. Based on these considerations, the Cys-to-Phe substitution was predicted to affect the proper folding of the domain and CBL function.

To validate the predicted impact of the p.Cys401Phe amino acid substitution on CBL function, COS-1 cells were transiently transfected with the HA-tagged CBL^{WT}, CBL^{C401F} and CBL^{R420Q} constructs, the latter included as representative of a CBL mutant that behave in a dominant-negative manner (Dunbar et al., 2008; Martinelli et al., 2010; Sargin et al., 2007). Similar to what has been observed for the CBL^{R420Q} mutant, albeit to a lower extent, western blot analysis showed enhanced EGF-induced ERK phosphorylation in cells overexpressing HA-CBL^{C401F} compared with cells expressing wild-type CBL (Figure 1b,c), validating a pathogenic role for this amino acid substitution. No gross effect of this mutant on AKT phosphorylation was noted.

4 | DISCUSSION

Protein ubiquitination is a post-translational reversible modification controlling a wide array of cellular processes, including signal transduction (Pickart & Eddins, 2004). In the context of RAS signaling, it is a well-recognized event contributing to signaling switch-off that is operated at different levels (Tartaglia et al., 2022). Different from LZTR1, which operates in a multi-protein complex implicated in the control of RIT/RAS function (Bigenzahn et al., 2018; Castel et al., 2019; Motta et al., 2019; Steklov et al., 2018), CBL is a single-RING E3 ubiquitin ligase that negatively regulates multiple cell surface RTKs, promoting their ubiquitination and subsequent degradation by the proteasome or via endocytosis. By functioning as an adaptor, CBL has also been reported to act as a positive regulator of signaling elicited by RTKs. Extensive studies demonstrated that E3 activation is required for RTKs ubiquitination (Martinelli et al., 2012; Niemeyer et al., 2010; Saettini et al., 2022; Thien et al., 2001). Multiple lines of evidence support the view that RASopathy- and leukemia-associated mutations affect CBL function and RTKs ubiquitination by impairing the E3 catalytic activity of the protein. The newly identified pathogenic variant (c.1202G>T; p.Cys401Phe) further supports this concept as it affects a residue located in the RING finger domain that has a crucial role in the maintenance of proper folding of this domain, and whose substitution results in an upregulation of the RAS-MAPK signaling cascade.

CBL syndrome is a highly variable condition accounting for <1% of subjects with some clinical features reminiscent of NS (Martinelli et al., 2010, 2015; Niemeyer et al., 2010; Pérez et al., 2010). Mild facial dysmorphisms, variable cardiac defects, ectodermal and musculoskeletal anomalies, DD and intellectual disability, brain MRI findings, and predisposition to JMML and cerebral vasculopathies characterize this condition. Different from the general NS population, *CBL* mutation-positive patients exhibit a lower prevalence of short stature (length below the 3rd centile; ~30% *vs.* ~90%) (Martinelli et al., 2015; Roberts et al., 2013), similar to what has been reported in individuals with *SOS1/SOS2* mutations (Cordeddu et al., 2015; Lepri et al., 2011). In line with this observation, growth parameters of the present subject were in the upper end of the normal range. Besides few typical features of NS, the proband showed some unusual findings, including ASD, JMML-free hepatosplenomegaly, recurrent infections, and hypertransaminasemia.

A large fraction of subjects harboring a germline *CBL* variant experience hematological and immunological problems (Roberts et al., 2013; Saettini et al., 2022). Hepatosplenomegaly, which is generally associated with JMML because of infiltration of leukemic cells into spleen and liver, may occur in CBL syndrome even in the absence of a clear myeloproliferative/myelodysplastic disorder (Ali et al., 2020; Becker et al., 2014; Coe et al., 2017; Leardini et al., 2022). In these individuals, however, as well as in the present subject, a subclinical episode of monocytosis or JMML-like myeloproliferative condition during infancy with spontaneous resolution cannot be ruled out. Germline *CBL* variants also predispose to a number of immunological issues, including autoimmune thrombocytopenia, rheumatoid arthritis, uveitis, and hemophagocytic syndrome secondary to EBV infection (Becker et al., 2014; Hadjadj et al., 2019; Pathak et al., 2015; Strullu et al., 2013; Tejwani et al., 2019; Wiel et al., 2020). A clinical overlap with autoimmune lymphoproliferative syndrome and B-cell expansion with NF-κB and T-cell anergy syndrome has been reported in some patients (Seaby et al., 2017), as well as the occurrence of early-onset moyamoya angiopathy (Guey et al., 2017). In this context, the thrombocytopenia and the recurrent hypertransaminasemia here observed may be of autoimmune origin, although we cannot exclude that elevated liver enzymes could be related to prolonged CMV infection in the first year of life. Finally, the prevalence of ASD in NS and other RASopathies is higher than expected (Adviento et al., 2014; Alfieri et al., 2014; Garg et al., 2017), which is not surprising given the role of the RAS-MAPK pathway in neurodevelopment (Garg & Green, 2018; Kim & Baek, 2019; Vithayathil et al., 2018).

In conclusion, the present findings further highlight that a clinically relevant *CBL* gene variant may be suspected in individuals with hematological and immunological manifestations in the presence of only minor signs of NS/RASopathy.

AUTHOR CONTRIBUTIONS

Functional studies: ES and LP. *Technical assistance*: SV. *Data collection, methodology, and writing*: JNC, DS, and DM. *Conceptualization, methodology, funding acquisition, writing, review, and editing*: JAB, MT, and SM.

ACKNOWLEDGMENTS

The authors thank the patient and his parents. Members of the Undiagnosed Diseases Network include: Maria T. Acosta, David R. Adams, Ben Afzali, Ali Al-Beshri, Eric Allenspach, Aimee Allworth, Raquel L. Alvarez, Justin Alvey, Ashley Andrews, Euan A. Ashley, Carlos A. Bacino, Guney Bademci, Ashok Balasubramanyam, Dustin Baldridge, Erin Baldwin, Jim Bale, Michael Bamshad, Deborah Barbouth, Pinar Bayrak-Toydemir, Anita Beck, Alan H. Beggs, Edward

Behrens, Gill Bejerano, Hugo J. Bellen, Jimmy Bennett, Jonathan A. Bernstein, Gerard T. Berry, Anna Bican, Stephanie Bivona, Elizabeth Blue, John Bohnsack, Devon Bonner, Nicholas Borja, Lorenzo Botto, Lauren C. Briere, Elizabeth A. Burke, Lindsay C. Burrage, Manish J. Butte, Peter Byers, William E. Byrd, Kaitlin Callaway, John Carey, George Carvalho, Thomas Cassini, Sirisak Chanprasert, Hsiao-Tuan Chao, Ivan Chinn, Gary D. Clark, Terra R. Coakley, Laurel A. Cobban, Joy D. Cogan, Matthew Coggins, F. Sessions Cole, Brian Corner, Rosario I. Corona, William J. Craigen, Andrew B. Crouse, Vishnu Cuddapah, Precilla D'Souza, Hongzheng Dai, Kahlen Darr, Surendra Dasari, Joie Davis, Margaret Delgado, Esteban C. Dell'Angelica, Katrina Dipple, Daniel Doherty, Naghmeh Dorrani, Jessica Douglas, Emilie D. Douine, Dawn Earl, Lisa T. Emrick, Christine M. Eng, Cecilia Esteves, Kimberly Ezell, Elizabeth L. Fieg, Paul G. Fisher, Brent L. Fogel, Jiayu Fu, William A. Gahl, Rebecca Ganetzky, Emily Glanton, Ian Glass, Page C. Goddard, Joanna M. Gonzalez, Andrea Gropman, Meghan C. Halley, Rizwan Hamid, Neal Hanchard, Kelly Hassey, Nichole Hayes, Frances High, Anne Hing, Fuki M. Hisama, Ingrid A. Holm, Jason Hom, Martha Horike-Pyne, Alden Huang, Yan Huang, Anna Hurst, Wendy Introne, Gail P. Jarvik, Suman Jayadev, Orpa Jean-Marie, Vaidehi Jobanputra, Oguz Kanca, Yigit Karasozen, Shamika Ketkar, Dana Kiley, Gonench Kilich, Eric Klee, Shilpa N. Kobren, Isaac S. Kohane, Jennefer N. Carter, Bruce Korf, Susan Korrick, Deborah Krakow, Elijah Kravets, Seema R. Lalani, Christina Lam, Brendan C. Lanpher, Ian R. Lanza, Kumarie Latchman, Kimberly LeBlanc, Brendan H. Lee, Kathleen A. Leppig, Richard A. Lewis, Pengfei Liu, Nicola Longo, Joseph Loscalzo, Richard L. Maas, Ellen F. Macnamara, Calum A. MacRae, Valerie V. Maduro, AudreyStephannie Maghiro, Rachel Mahoney, May Christine V. Malicdan, Rong Mao, Ronit Marom, Gabor Marth, Beth A. Martin, Martin G. Martin, Julian A. Martínez-Agosto, Shruti Marwaha, Allyn McConkie-Rosell, Ashley McMinn, Matthew Might, Mohamad Mikati, Danny Miller, Ghayda Mirzaa, Breanna Mitchell, Paolo Moretti, Marie Morimoto, John J. Mulvihill, Lindsay Mulvihill, Mariko Nakano-Okuno, Stanley F. Nelson, Serena Neumann, Dargie Nitsuh, Donna Novacic, Devin Oglesbee, James P. Orengo, Laura Pace, Stephen Pak, J. Carl Pallais, Neil H. Parker, LéShon Peart, Leoyklang Petcharet, John A. Phillips III, Filippo Pinto e Vairo, Jennifer E. Posey, Lorraine Potocki, Barbara N. Pusey Swerdzewski, Aaron Quinlan, Daniel J. Rader, Ramakrishnan Rajagopalan, Deepak A. Rao, Anna Raper, Wendy Raskind, Adriana Rebelo, Chloe M. Reuter, Lynette Rives, Lance H. Rodan, Martin Rodriguez, Jill A. Rosenfeld, Elizabeth Rosenthal, Francis Rossignol, Maura Ruzhnikov, Marla Sabaii, Jacinda B. Sampson, Timothy Schedl, Lisa Schimmenti, Kelly Schoch, Daryl A. Scott, Elaine Seto, Vandana Shashi, Emily Shelkowitz, Sam Sheppeard, Jimann Shin, Edwin K. Silverman, Giorgio Sirugo, Kathy Sisco, Tammi Skelton, Cara Skraban, Carson A. Smith, Kevin S. Smith, Lilianna Solnica-Krezel, Ben Solomon, Rebecca C. Spillmann, Andrew Stergachis, Joan M. Stoler, Kathleen Sullivan, Shamil R. Sunyaev, Shirley Sutton, David A. Sweetser, Virginia Sybert, Holly K. Tabor, Queenie Tan, Arjun Tarakad, Herman Taylor, Mustafa Tekin, Willa Thorson, Cynthia J. Tifft, Camilo Toro, Alyssa A. Tran, Rachel A. Ungar, Adeline Vanderver, Matt Velinder, Dave Viskochil, Tiphanie P. Vogel, Colleen E. Wahl, Melissa Walker, Nicole M. Walley, Jennifer Wambach, Michael F. Wangler, Patricia A. Ward, Daniel Wegner, Monika Weisz Hubshman, Mark Wener, Tara Wenger, Monte Westerfield, Matthew T. Wheeler, Jordan Whitlock, Lynne A. Wolfe, Heidi Wood, Kim Worley, Shinya Yamamoto, Zhe Zhang, Stephan Zuchner.

FUNDING INFORMATION

Research reported in this manuscript was supported by the NIH Common Fund, through the Office of Strategic Coordination/Office of the NIH Director under Award Number U01HG010218, Associazione Italiana per la Ricerca sul Cancro, AIRC (IG 28768), Italian Ministry of Health (5 per 1000_2024 and Current Research Funding) and European Joint Programme on Rare Diseases, EJP-RD (NSEuroNet). The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

CONFLICT OF INTEREST STATEMENT

The authors have no relevant conflicts of interest to report.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

PATIENT CONSENT

Patient's parents gave informed written consent.

ORCID

David Stevenson https://orcid.org/0000-0001-8601-0020
Jonathan A. Bernstein https://orcid.org/0000-0001-5369-346X
Marco Tartaglia https://orcid.org/0000-0001-7736-9672
Simone Martinelli https://orcid.org/0000-0002-5843-419X

REFERENCES

Adviento, B., Corbin, I. L., Widjaja, F., Desachy, G., Enrique, N., Rosser, T., Risi, S., Marco, E. J., Hendren, R. L., Bearden, C. E., Rauen, K. A., & Weiss, L. A. (2014). Autism traits in the RASopathies. *Journal of Medical Genetics*, *51*(1), 10–20. https://doi.org/10.1136/jmedgenet-2013-101951

Alfieri, P., Piccini, G., Caciolo, C., Perrino, F., Gambardella, M. L., Mallardi, M., Cesarini, L., Leoni, C., Leone, D., Fossati, C., Selicorni, A., Digilio, M. C., Tartaglia, M., Mercuri, E., Zampino, G., & Vicari, S. (2014). Behavioral profile in RASopathies. *American Journal of Medical Genetics Part A*, *164A*(4), 934–942. https://doi.org/10.1002/ajmg.a.36374

Ali, A. M., Cooper, J., Walker, A., Jones, D., & Saad, A. (2020). Adult-onset acute myeloid leukaemia in a patient with germline mutation of CBL. *British Journal of Haematology*, *192*(3), 665–667. https://doi.org/10.1111/bjh.17234

Becker, H., Yoshida, K., Blagitko-Dorfs, N., Claus, R., Pantic, M., Abdelkarim, M., Niemöller, C., Greil, C., Hackanson, B., Shiraishi, Y., Chiba, K., Tanaka, H., Miyano, S., Döhner, K., Schnittger, S., Henneke, P., Niemeyer, C. M., Flotho, C., Pfeifer, D., … Lübbert, M. (2014). Tracing the development of acute myeloid leukemia in CBL syndrome. *Blood*, *123*(12), 1883–1886. https://doi.org/10.1182/blood-2013-10-533844

Belizaire, R., Koochaki, S. H. J., Udeshi, N. D., Vedder, A., Sun, L., Svinkina, T., Hartigan, C., McConkey, M., Kovalcik, V., Bizuayehu, A., Stanclift, C., Schenone, M., Carr, S. A., Padron, E., & Ebert, B. L. (2022). CBL mutations drive PI3K/AKT signaling via increased interaction with LYN and PIK3R1. *Blood*, *137*(16), 2209–2220. https://doi.org/10.1182/blood.2020006528

Bigenzahn, J. W., Collu, G. M., Kartnig, F., Pieraks, M., Vladimer, G. I., Heinz, L. X., Sedlyarov, V., Schischlik, F., Fauster, A., Rebsamen, M., Parapatics, K., Blomen, V. A., Müller, A. C., Winter, G. E., Kralovics, R., Brummelkamp, T. R., Mlodzik, M., & Superti-Furga, G. (2018). LZTR1 is a regulator of RAS ubiquitination and signaling. *Science*, *362*(6419), 1171–1177. https://doi.org/10.1126/science.aap8210

Borja, N., Bivona, S., Peart, L. S., Johnson, B., Gonzalez, J., Barbouth, D., Moore, H., Guo, S., Undiagnosed Disease Network, Bademci, G., & Tekin, M. (2022). Genome sequencing reveals novel noncoding variants in PLA2G6 and LMNB1 causing progressive neurologic disease. *Molecular Genetics & Genomic Medicine*, *10*(4), e1892. https://doi.org/10.1002/mgg3.1892

Castel, P., Cheng, A., Cuevas-Navarro, A., Everman, D. B., Papageorge, A. G., Simanshu, D. K., Tankka, A., Galeas, J., Urisman, A., & McCormick, F. (2019). RIT1 oncoproteins escape LZTR1-mediated proteolysis. *Science*, *363*(6432), 1226–1230. https://doi.org/10.1126/science.aav1444

Coe, R. R., McKinnon, M., Tarailo-Graovac, M., Ross, C. J., Wasserman, W., Friedman, J. M., Rogers, P. C., & Van Karnebeek, C. D. (2017). A case of splenomegaly in CBL syndrome. *European Journal of Medical Genetics*, *60*(7), 374–379. https://doi.org/10.1016/j.ejmg.2017.04.009

Cordeddu, V., Yin, J. C., Gunnarsson, C., Virtanen, C., Drunat, S., Lepri, F., De Luca, A., Rossi, C., Ciolfi, A., Pugh, T. J., Bruselles, A., Priest, J. R., Pennacchio, L. A., Lu, Z., Danesh, A., Quevedo, R., Hamid, A., Martinelli, S., Pantaleoni, F., … Tartaglia, M. (2015). Activating mutations affecting the Dbl homology domain of SOS2 cause Noonan syndrome. *Human Mutation*, *36*(11), 1080–1087. https://doi.org/10.1002/humu.22834

Dikic, I., & Schmidt, M. H. H. (2007). Malfunctions within the Cbl interactome uncouple receptor tyrosine kinases from destructive transport. *European Journal of Cell Biology*, *86*(9), 505–512. https://doi.org/10.1016/j.ejcb.2007.04.005

Dou, H., Buetow, L., Hock, A., Sibbet, G. J., Vousden, K. H., & Huang, D. T. (2012). Structural basis for autoinhibition and phosphorylation-dependent activation of c-Cbl. *Nature Structural & Molecular Biology*, *19*, 184–192. https://doi.org/10.1038/nsmb.2231

Dunbar, A. J., Gondek, L. P., O'Keefe, C. L., Makishima, H., Rataul, M. S., Szpurka, H., Sekeres, M. A., Wang, X. F., McDevitt, M. A., & Maciejewski, J. P. (2008). 250K single nucleotide polymorphism array karyotyping identifies acquired uniparental disomy and homozygous mutations, including novel missense substitutions of c-Cbl, in myeloid malignancies. *Cancer Research*, *68*(24), 10349–10357. https://doi.org/10.1158/0008-5472.CAN-08-2754

Garg, S., Brooks, A., Burns, A., Burkitt-Wright, E., Kerr, B., Huson, S., Emsley, R., & Green, J. (2017). Autism spectrum disorder and other neurobehavioural comorbidities in rare disorders of the Ras/MAPK pathway. *Developmental Medicine and Child Neurology*, *59*(5), 544–549. https://doi.org/10.1111/dmcn.13394

Garg, S., & Green, J. (2018). Studying child development in genetic models of ASD. *Progress in Brain Research*, *241*, 159–192. https://doi.org/10.1016/bs.pbr.2018.09.009

Guey, S., Grangeon, L., Brunelle, F., Bergametti, F., Amiel, J., Lyonnet, S., Delaforge, A., Arnould, M., Desnous, B., Bellesme, C., Hervé, D., Schwitalla, J. C., Kraemer, M., Tournier-Lasserve, E., & Kossorotoff, M. (2017). De novo mutations in CBL causing early-onset paediatric moyamoya angiopathy. *Journal of Medical Genetics*, *54*(8), 550–557. https://doi.org/10.1136/jmedgenet-2016-104432

Hadjadj, J., Aladjidi, N., Fernandes, H., Leverger, G., Magérus-Chatinet, A., Mazerolles, F., Stolzenberg, M. C., Jacques, S., Picard, C., Rosain, J., Fourrage, C., Hanein, S., Zarhrate, M., Pasquet, M., Chahla, W. A., Barlogis, V., Bertrand, Y., Pellier, I., Colomb Bottollier, E., … Members of the French Reference Center for Pediatric Autoimmune Cytopenia (CEREVANCE). (2019). Pediatric Evans syndrome is associated with a high frequency of potentially damaging variants in immune genes. *Blood*, *134*(1), 9–21. https://doi.org/10.1182/blood-2018-11-887141

Hecht, A., Meyer, J. A., Behnert, A., Wong, E., Chehab, F., Olshen, A., Hechmer, A., Aftandilian, C., Bhat, R., Choi, S. W., Chonat, S., Farrar, J. E., Fluchel, M., Frangoul, H., Han, J. H., Kolb, E. A., Kuo, D. J., MacMillan, M. L., Maese, L., … Stieglitz, E. (2022). Molecular and phenotypic diversity of CBL-mutated juvenile myelomonocytic leukemia. *Haematologica*, *107*(1), 178–186. https://doi.org/10.3324/haematol.2020.270595

Joazeiro, C. A., Wing, S. S., Huang, H., Leverson, J. D., Hunter, T., & Liu, Y. C. (1999). The tyrosine kinase negative regulator c-Cbl as a RING-type, E2-dependent ubiquitin-protein ligase. *Science*, *286*(5438), 309–312. https://doi.org/10.1126/science.286.5438.309

Kales, S. C., Ryan, P. E., Nau, M. M., & Lipkowitz, S. (2010). Cbl and human myeloid neoplasms: The Cbl oncogene comes of age. *Cancer Research*, *70*(12), 4789–4794. https://doi.org/10.1158/0008-5472.CAN-10-0610

Kim, Y. E., & Baek, S. T. (2019). Neurodevelopmental aspects of RASopathies. *Molecules and Cells*, *42*(6), 441–447. https://doi.org/10.14348/molcells.2019.0037

Leardini, D., Messelodi, D., Muratore, E., Baccelli, F., Bertuccio, S. N., Anselmi, L., Pession, A., & Masetti, R. (2022). Role of CBL mutations in cancer and non-malignant phenotype. *Cancers*, *14*(3), 839. https://doi.org/10.3390/cancers14030839

Lepri, F., De Luca, A., Stella, L., Rossi, C., Baldassarre, G., Pantaleoni, F., Cordeddu, V., Williams, B. J., Dentici, M. L., Caputo, V., Venanzi, S., Bonaguro, M., Kavamura, I., Faienza, M. F., Pilotta, A., Stanzial, F., Faravelli, F., Gabrielli, O., Marino, B., … Tartaglia, M. (2011). SOS1 mutations in Noonan syndrome: Molecular spectrum, structural insights on pathogenic effects, and genotype-phenotype correlations. *Human Mutation*, *32*(7), 760–772. https://doi.org/10.1002/humu.21492

Martinelli, S., Checquolo, S., Consoli, F., Stellacci, E., Rossi, C., Silvano, M., Franciosa, G., Flex, E., Cossu, C., De Luca, A., Foà, R., Cazzaniga, G., Biondi, A., Screpanti, I., & Tartaglia, M. (2012). Loss of CBL E3-ligase activity in B-lineage childhood acute lymphoblastic leukaemia. *British Journal of Haematology*, *159*(1), 115–119. https://doi.org/10.1111/j.1365-2141.2012.09245.x

Martinelli, S., De Luca, A., Stellacci, E., Rossi, C., Checquolo, S., Lepri, F., Caputo, V., Silvano, M., Buscherini, F., Consoli, F., Ferrara, G., Digilio, M. C., Cavaliere, M. L., Van Hagen, J. M., Zampino, G., Van der Burgt, I., Ferrero, G. B., Mazzanti, L., Screpanti, I., … Tartaglia, M. (2010). Heterozygous germline mutations in the *CBL* tumor-suppressor gene cause a Noonan syndrome-like phenotype. *American Journal of Human Genetics*, *87*(2), 250–257. https://doi.org/10.1016/j.ajhg.2010.06.015

Martinelli, S., Stellacci, E., Pannone, L., D'Agostino, D., Consoli, F., Lissewski, C., Silvano, M., Cencelli, G., Lepri, F., Maitz, S., Pauli, S., Rauch, A., Zampino, G., Selicorni, A., Melançon, S., Digilio, M. C., Gelb, B. D., De Luca, A., Dallapiccola, B., … Tartaglia, M. (2015). Molecular diversity and associated phenotypic spectrum of germline CBL mutations. *Human Mutation*, *36*(8), 787–796. https://doi.org/10.1002/humu.22809

Motta, M., Fidan, M., Bellacchio, E., Pantaleoni, F., Schneider-Heieck, K., Coppola, S., Borck, G., Salviati, L., Zenker, M., Cirstea, I. C., & Tartaglia, M. (2019). Dominant Noonan syndrome-causing LZTR1 mutations specifically affect the Kelch domain substrate-recognition surface and enhance RAS-MAPK signaling. *Human Molecular Genetics*, *28*(6), 1007–1022. https://doi.org/10.1093/hmg/ddy412

Muramatsu, H., Makishima, H., Jankowska, A. M., Cazzolli, H., O'Keefe, C., Yoshida, N., Xu, Y., Nishio, N., Hama, A., Yagasaki, H., Takahashi, Y.,

Kato, K., Manabe, A., Kojima, S., & Maciejewski, J. P. (2010). Mutations of an E3 ubiquitin ligase c-Cbl but not TET2 mutations are pathogenic in juvenile myelomonocytic leukemia. *Blood*, *115*(10), 1969–1975. https://doi.org/10.1182/blood-2009-06-226340

Niemeyer, C. M., Kang, M. W., Shin, D. H., Furlan, I., Erlacher, M., Bunin, N. J., Bunda, S., Finklestein, J. Z., Gorr, T. A., Mehta, P., Schmid, I., Kropshofer, G., Corbacioglu, S., Lang, P. J., Klein, C., Schlegel, P. G., Heinzmann, A., Schneider, M., Starý, J., … Loh, M. L. (2010). Germline *CBL* mutations cause developmental abnormalities and predispose to juvenile myelomonocytic leukemia. *Nature Genetics*, *42*(9), 794–800. https://doi.org/10.1038/ng.641

Ogawa, S., Shih, L. Y., Suzuki, T., Otsu, M., Nakauchi, H., Koeffler, H. P., & Sanada, M. (2010). Deregulated intracellular signaling by mutated c-CBL in myeloid neoplasms. *Clinical Cancer Research*, *16*(15), 3825–3831. https://doi.org/10.1158/1078-0432.CCR-09-2341

Ota, S., Hazeki, K., Rao, N., Lupher, M. L., Jr., Andoniou, C. E., Druker, B., & Band, H. (2000). The RING finger domain of Cbl is essential for negative regulation of the Syk tyrosine kinase. *Journal of Biological Chemistry*, *275*(1), 414–422. https://doi.org/10.1074/jbc.275.1.414

Pathak, A., Pemov, A., McMaster, M. L., Dewan, R., Ravichandran, S., Pak, E., Dutra, A., Lee, H. J., Vogt, A., Zhang, X., Yeager, M., Anderson, S., Kirby, M., NCI DCEG Cancer Genomics Research Laboratory, NCI DCEG Cancer Sequencing Working Group, Caporaso, N., Greene, M. H., Goldin, L. R., & Stewart, D. R. (2015). Juvenile myelomonocytic leukemia due to a germline CBL Y371C mutation: 35-year follow-up of a large family. *Human Genetics*, *134*(7), 775–787. https://doi.org/10.1007/s00439-015-1550-9

Pérez, B., Mechinaud, F., Galambrun, C., Ben Romdhane, N., Isidor, B., Philip, N., Derain-Court, J., Cassinat, B., Lachenaud, J., Kaltenbach, S., Salmon, A., Désirée, C., Pereira, S., Menot, M. L., Royer, N., Fenneteau, O., Baruchel, A., Chomienne, C., Verloes, A., & Cavé, H. (2010). Germline mutations of the *CBL* gene define a new genetic syndrome with predisposition to juvenile myelomonocytic leukaemia. *Journal of Medical Genetics*, *47*(10), 686–691. https://doi.org/10.1136/jmg.2010.076836

Pickart, C. M., & Eddins, M. J. (2004). Ubiquitin: Structures, functions, mechanisms. *Biochimica et Biophysica Acta*, *1695*(1), 55–72. https://doi.org/10.1016/j.bbamcr.2004.09.019

Richards, S., Aziz, N., Bale, S., Bick, D., Das, S., Gastier-Foster, J., Grody, W. W., Hegde, M., Lyon, E., Spector, E., Voelkerding, K., Rehm, H. L., Committee, A., & ACMG Laboratory Quality Assurance Committee. (2015). Standards and guidelines for the interpretation of sequence variants: A joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genetics in Medicine*, *17*(5), 405–423. https://doi.org/10.1038/gim.2015.30

Roberts, A. E., Allanson, J. E., Tartaglia, M., & Gelb, B. D. (2013). Noonan syndrome. *Lancet*, *381*(9863), 333–342. https://doi.org/10.1016/S0140-6736(12)61023-X

Saettini, F., Coliva, T. A., Vendemini, F., Galbiati, M., Bugarin, C., Masetti, R., Moratto, D., Chiarini, M., Guerra, F., Iascone, M., Badolato, R., Cazzaniga, G., Niemeyer, C., Flotho, C., & Biondi, A. (2022). Abnormal B-cell maturation and increased transitional B cells in CBL syndrome. *Frontiers in Pediatrics*, *10*, 935951. https://doi.org/10.3389/fped.2022.935951

Sanada, M., Suzuki, T., Shih, L. Y., Otsu, M., Kato, M., Yamazaki, S., Tamura, A., Honda, H., Sakata-Yanagimoto, M., Kumano, K., Oda, H., Yamagata, T., Takita, J., Gotoh, N., Nakazaki, N., Kawamata, N., Onodera, M., Nobuyoshi, M., Hayashi, Y., … Ogawa, S. (2009). Gain-of-function of mutated C-CBL tumour suppressor in myeloid neoplasms. *Nature*, *460*(7257), 904–908. https://doi.org/10.1038/nature08240

Sargin, B., Choudhary, C., Crosetto, N., Schmidt, M. H. H., Grundler, R., Rensinghoff, M., Rensinghoff, M., Thiessen, C., Tickenbrock, L., Schwäble, J., Brandts, C., August, B., Koschmieder, S., Rao Bandi, S., Duyster, J., Berdel, W. E., Müller-Tidow, C., Dikic, I., & Serve, H. (2007). Flt3-dependent transformation by inactivating c-Cbl mutations in AML. *Blood*, *110*(3), 1004–1012. https://doi.org/10.1182/blood-2007-01-066076

Schmidt, M. H., & Dikic, I. (2005). The Cbl interactome and its functions. *Nature Reviews Molecular Cell Biology*, *6*, 907–918. https://doi.org/10.1038/nrm1762

Seaby, E. G., Gilbert, R. D., Andreoletti, G., Pengelly, R. J., Mercer, C., Hunt, D., & Ennis, S. (2017). Unexpected findings in a child with atypical hemolytic uremic syndrome: An example of how genomics is changing the clinical diagnostic paradigm. *Frontiers in Pediatrics*, *5*, 113. https://doi.org/10.3389/fped.2017.00113

Steklov, M., Pandolfi, S., Baietti, M. F., Batiuk, A., Carai, P., Najm, P., Zhang, M., Jang, H., Renzi, F., Cai, Y., Abbasi Asbagh, L., Pastor, T., De Troyer, M., Simicek, M., Radaelli, E., Brems, H., Legius, E., Tavernier, J., Gevaert, K., … Sablina, A. A. (2018). Mutations in LZTR1 drive human disease by dysregulating RAS ubiquitination. *Science*, *362*(6419), 1177–1182. https://doi.org/10.1126/science.aap7607

Strullu, M., Caye, A., Cassinat, B., Fenneteau, O., Touzot, F., Blauwblomme, T., Rodriguez, R., Latour, S., Petit, A., Barlogis, V., Galambrun, C., Leblanc, T., Baruchel, A., Chomienne, C., & Cavé, H. (2013). In hematopoietic cells with a germline mutation of CBL, loss of heterozygosity is not a signature of juvenile myelo-monocytic leukemia. *Leukemia*, *27*(12), 2404–2407. https://doi.org/10.1038/leu.2013.203

Swaminathan, G., & Tsygankov, A. Y. (2006). The Cbl family proteins: Ring leaders in regulation of cell signaling. *Journal of Cellular Physiology*, *209*(1), 21–43. https://doi.org/10.1002/jcp.20694

Tartaglia, M., Aoki, Y., & Gelb, B. D. (2022). The molecular genetics of RASopathies: An update on novel disease genes and new disorders. *American Journal of Medical Genetics. Part C, Seminars in Medical Genetics*, *190*, 425–439. https://doi.org/10.1002/ajmg.c.32012

Tartaglia, M., & Gelb, B. D. (2010). Disorders of dysregulated signal traffic through the RAS-MAPK pathway: Phenotypic spectrum and molecular mechanisms. *Annals of the New York Academy of Sciences*, *1214*, 99–121. https://doi.org/10.1111/j.1749-6632.2010.05790.x

Tejwani, N., Tayal, P., Mehta, A., & Dass, J. (2019). Somatic hemizygous Y371H CBL mutation with loss of heterozygosity presenting with BENTA type lymphoid proliferation. *Indian Journal of Hematology and Blood Transfusion*, *36*(3), 594–596. https://doi.org/10.1007/s12288-019-01243-1

Thien, C. B., Walker, F., & Langdon, W. Y. (2001). RING finger mutations that abolish c-Cbl-directed polyubiquitination and downregulation of the EGF receptor are insufficient for cell transformation. *Molecular Cell*, *7*(2), 355–365. https://doi.org/10.1016/s1097-2765(01)00183-6

Vithayathil, J., Pucilowska, J., & Landreth, G. E. (2018). ERK/MAPK signaling and autism spectrum disorders. *Progress in Brain Research*, *241*, 63–112. https://doi.org/10.1016/bs.pbr.2018.09.008

Wiel, C. L., Pastore, S., Taddio, A., & Tommasini, A. (2020). A case of uveitis in a patient with juvenile myelomonocytic leukemia successfully treated with adalimumab. *Journal of Pediatric Hematology/Oncology*, *42*(5), e373–e376. https://doi.org/10.1097/MPH.0000000000001448

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Stellacci, E., Carter, J. N., Pannone, L., Stevenson, D., Moslehi, D., Venanzi, S., Undiagnosed Diseases Network, Bernstein, J. A., Tartaglia, M., & Martinelli, S. (2024). Immunological and hematological findings as major features in a patient with a new germline pathogenic *CBL* variant. *American Journal of Medical Genetics Part A*, *194*A:e63627. https://doi.org/10.1002/ajmg.a.63627